UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

005055

4/23/1986

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPTC-RS-DCI. Evaluation of Two-Year Chronic Rat Study (Accession Nos. 254335, 254336, 254337, 254338, and Addendum to Final Report (Accession Nos. 258076, 260057).

Caswell No. 435
EPA ID 476-2140

FROM: Irving Mauer, Ph.D.
Toxicology Branch
Hazard Evaluation Division (TS-769C)

04-15-86

TO: Robert J. Taylor, PM 25
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and

James R. Yowell, PM 25
Fungicide-Herbicide Branch
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THRU: Jane E. Harris, Ph.D., Head
Section VI, Toxicology Branch
Hazard Evaluation Division (TS-769C)

JEH 4/15/86

4/23/86

Registrant: Stauffer Chemical Company

Action Requested: (661). Review the following study submitted by the registrant in response to the Data Call-In Notice for Eptam:

Two-Year Oral Toxicity/Oncogenicity Study in Rats with R-1608 (EPTAM®), Study No. T-10001, dated July 27, 1984, conducted by IRDC (Accession Nos. 254335 to 254338).

Addendum, dated May 24, 1985. (Accession Nos. 258076, 260057 - both copy #2 of the same data.)

Toxicology Branch Conclusions: The study has been graded CORE-MINIMUM data for both chronic toxicity and oncogenicity (see Data Review attached to this memorandum).

At 25 mg/kg/day and above, decreased body weight gain and food consumption, and axonal degeneration and muscle atrophy were observed. At the highest dose tested, 125 mg/kg/day, the following effects were also present: chronic myocarditis (combined with organized atrial thrombosis in high-dose females dying-on-study); cataracts in high-dose females; increased SGOT activity (which correlates with cardiac changes); and decreased erythrocyte AChE activity. The data support a NOEL of 5 mg/kg/day (LDT).

No induced tumors at the highest dose tested, at which level increased mortality was observed.

Attachment

TOXICOLOGY BRANCH: DATA REVIEW

Chemical: EPTC

Caswell No. 435
Chem. No. 041401

Study Type: Chronic (2-year) oral (feeding) Toxicity/Oncogenicity - Rat

Citation: Two-Year Oral Toxicity/Oncogenicity Study in Rats With R-1608 (EPTAM®)/and Addendum

Accession Nos.: 254335, 254336, 254337, 254338/258076, 260057

Sponsor/Testing Lab: Stauffer Chemical/International Research and Development Corporation (IRDC)

Study No./Date: T-10001/September 30, 1983 (IRDC). Submitted by Stauffer July 27, 1984 (Project No. 142281). Addendum May 24, 1985.

Test Material: EPTAM Technical (Lot #CHK-0601/WRC #4291-4-10), a pale amber liquid (percent purity not stated in the report).

Procedures:

A copy of the Materials and Methods is attached to this review as Appendix A. The study was reportedly conducted according to FIFRA Guidelines subsections 83-1/83-2 and FDA-GLP's. Briefly, male and female Charles River CD rats (60/sex/group) were fed diets (prepared weekly) containing EPTC to provide intakes of 0, 5, 25, and 125 mg/kg/day (adjusted weekly, based upon body weight and food consumption). Rats were observed twice daily for overt signs of clinical toxicity, and detailed observations were conducted weekly. Baseline clinical lab values were determined for 20 rats/sex during the pretest period, as well as periodically during the study on 10 rats/sex/group (at 6, 12, and 18 months), and on 20 animals/sex/group at termination (24 months). Ophthalmoscopic examinations were performed on all animals surviving 26, 51, 78, and 104 weeks. Postmortem examination on all animals (dying on study, interim 12 months, and all survivors to 24 months) included the full registry of organs and tissues prescribed by the Guidelines (see Tissue Inventory, Report Appendix I); protocol-designated tissues from control and high-dose groups were processed for histopathological examination (including three coronal sections of the head from 10 rats/sex/group surviving to 24 months). In addition, neuromuscular tissues from the 5 and 25 mg/kg/day dosage

groups (sciatic and tibial nerves, their associated musculature, biceps femoris, and spinal cord sections from thoracic lumbar and sacral regions) were identified as target organs, and hence also examined (see Appendix H).

The following standard (referenced) statistical procedures were used to analyze the data for body weight, absolute and relative organ weights, and hematological, biochemical, and cholinesterase values: Analysis of variance (one-way classification), Bartlett's test for homogeneity of variances and the appropriate t-test (for equal or unequal variances) as described by Steel and Torrie and Ostle, using Dunnett's multiple comparison tables to judge significance of differences between treatment groups and controls.

Complete methods for diet analysis from weekly sample collections were provided in the report. (See Report pages 13 and 14, attached as Appendix A to this review.)

Results:

During the first 6 months, seven high-dose males died (Report Table 3), six of the seven during study weeks 22 to 24 (of a variety of reported causes, presumably complicated by interstitial pneumonia/inflammation, as recorded microscopically in Report Table 11). A further four high-dose males were found dead before the scheduled 1-year interim sacrifice (during weeks 27, 29, 43, and 47, as recorded in Appendix H of the report).* Mortality among low- and mid-dose males, as well as among all female test groups, did not differ from their respective controls during the first year of the study.** Increased mortality was also recorded among high-dose males (but not in the high-dose female group) late in the second year of the study (between weeks 98 and 104), a period when a total of eight animals were found dead (compared to a steady rate of death of one to three males during prior 4-week periods--Report Table 3). Survival at study termination was summarized in the report as follows (page 16 of the Report):

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*NB: Of the 11 males in the highest dose group that died within the first year on test, 3 showed microscopic finding of cardiac mononuclear cell infiltration while 3 others had chronic myocarditis, as noted by the reviewer in Table 11 and Appendix H. Chronic myocarditis was also recorded as a microscopic finding in 4 of 10 high-dose males sacrificed after 1 year on study (Table 11, Appendix H).

**Microscopic evidence of chronic myocarditis was recorded in 5 of 10 high-dose females sacrificed at 1 year (but not in two high-dose females found dead during weeks 25 and 38), as identified in Appendix H of the report and summarized in Table 11.

Dosage Level (mg/kg/day)	Number Surviving/ Number Assigned (Less Interim Sacrifice) Male	Female
0 (control)	29/50	30/50
5	32/50	29/50
25	29/50	27/50
125*	15/50	26/50

Dose-related decreases in body weight (Report Tables 2 and 3, Graphs 1 and 2, Appendix C) were found beginning as early as study week 5 in the high-dose group and becoming progressively more severe in this group throughout the remainder of the study. Statistically significant body weight depressions also occurred at the mid-dose level, beginning a little later (weeks 13 to 14), but only reaching a level of toxicologic significance during the second year of the study, whereas at the low dose, only occasional statistically significant decreases were noted. Corresponding dose-related reductions in food consumption were also recorded throughout the study (Table 4, Graphs 3 and 4, Appendix D). Mean ponderal and feed consumption values at termination were summarized by the investigators as follows:

Dosage Level mg/kg/day	Body Weight, g (% difference from control)		Food Consumption g/rat/day (% diff. from control)	
	MALE	FEMALE	MALE	FEMALE
0 (control)	792	517	26.8	19.7
5	739 (-6.7)	465 (-10.1)	24.8 (-7.5)	19.0 (-3.6)
25	672 (-15.2)	435 (-15.9)	24.5 (-8.9)	18.7 (-5.1)
125	507 (-36.0)	312 (-39.7)	22.5 (-16.0)	16.9 (-14.2)

Periodic test diet analyses throughout the study (Appendix K) indicated that compound concentrations were 78 to 107 percent of target test article amounts; reported compound consumption over the 104 weeks for the three test groups averaged, respectively (males/females), 5.01/4.97, 25.0/24.8, and 125.8/124.8, at the low-, mid-, and high-dose levels.

*According to Appendices H and L of the Report, however, only 14 males and 24 females survived to the scheduled terminal sacrifice.

Ophthalmoscopic examinations (Table 8, Appendix I) revealed a high incidence of cataractous changes late in the study (104 weeks) among high-dose females (13/23 vs. 2/28 for controls), considered by the authors as suggestive of a compound-related effect. Other signs assumed to be compound related were listed as (Table 1, Appendix B): (1) Discolored urine (described as red to reddish brown), primarily in high-dose males*; (2) "testes located in a posterior position," becoming evident toward the middle of the second year at 125 mg/kg/day, at which time a few animals at 25 mg/kg/day were noted to display this effect; and (3) hindquarters weakness ("inability to assume an upright posture bearing the weight on the plantar surface of the hindfeet"), also observed late in the study (weeks 85 to 95 ff), first among the high-dose female group, but shortly thereafter also in a few high-dose males.

The slight but statistically significant differences recorded between some control and EPTC-treated mean hematological values (Table 5, Appendix E) were considered by the authors to be without biological significance, but rather due to normal variability. More consistent differences between high-dose males and controls for activated partial thromboplastin time (APTT) and prothrombin time (PT), however, were seen frequently (at every sampling from 6 months on--as recorded in Table 5), but the authors offered no explanation for these increases.

Clinical chemistry values were generally similar between control and test groups for both sexes (Table 6, Appendix F). The occasional significant differences observed during the study (e.g., increases in BUN and SGOT activity in high-dose males and females) were considered by the authors to reflect normal biological variability.** Decreased erythrocyte cholinesterase activity (but not plasma or brain ChE) was recorded in high-dose females and males.

At terminal sacrifice, significant group differences in absolute and/or relative organ weights were recorded in animals dosed at 25 and 125 mg/kg/day ($p < 0.01$ at the high-dose level). As summarized in Table 10 of the report (from individual values in Appendix G), the affected organs included (for both sexes):

*Hemorrhage and/or cystitis was observed microscopically in urinary bladders of some males which died on study (but not in scheduled sacrifices) (tables 11, 12), which may explain the coloration; however, no urinalysis data were provided to confirm the possibility of bloody discharge.

**However, increased SGOT activity can be correlated with increased cardiac lesions at the highest dose.

liver, lung, brain, kidney, and heart. These variations were considered by the authors to reflect reductions in mean body weight which occurred at these levels, and not toxicologically significant. Further support for this assessment was the assertion that no compound-related lesions were macroscopically evident in any of these major organs.*

Microscopic examination of all inventoried tissues at the 1-year interim sacrifice was stated to be negative for compound-related changes (as referenced in Table 11 and Appendix H--but see reviewer's footnotes, page 3 of this review). In terminally sacrificed animals and those that died during the second year of the study, however, the following syndrome of neuromuscular lesions was reported (as referenced in Table 12 and Appendix I of the report), consisting of (as quoted from page 21 of the report):

1. "Increased incidence of atrophy or degeneration in the muscle adjacent to the sciatic nerve and in the biceps femoris muscle in male and female rats from the 25 and 125 mg/kg/day groups.

2. "Atrophy or increased incidence of axonal degeneration in sciatic and tibial nerves of male and female rats from the 25 and 125 mg/kg/day groups.

3. "A slight increase in the incidence of axonal degeneration in lumbar spinal cord in male and female rats from the 25 and 125 mg/kg/day groups. In most instances, this change was more evident in nerve roots emerging from the concerned cord."

A detailed summary of the incidence of these lesions provided in the report (pages 21 and 22, and reprinted on the review page immediately following) revealed only a low incidence of trace-to-mild degeneration in muscle adjacent to the sciatic nerve and biceps femoris in low-dose rats, which the authors considered "spontaneous in nature." Further, the low and/or comparable incidence of axonal degeneration in thoracic and sacral spinal cords suggested to the authors these changes were also not compound-related, since ". . . spontaneous occurrence of axonal degeneration in spinal cord, especially in the nerve roots from the cord, was not uncommon in aging rats." On the other hand, the authors offered the high incidence of atrophy and axonal degeneration of sciatic and tibial nerves (resulting in degeneration or atrophy of associated musculature of hind limbs) as possibly (probably?) explaining the impaired function of hind

*However, see below, concerning heart lesions (TB Evaluation).

limbs and inability to maintain weight on hindleg plantar surfaces observed in high-dose males and females late in the study (beginning about week 85).

Incidence of Neuromuscular Lesions In All Rats 12 to 24 Months On Study*

mg/kg/day	MALES 0	5	25	125	FEMALES 0	5	25	125
MUSCLE, ADJACENT TO SCIATIC NERVE	(46)	(49)	(47)	(39)	(47)	(46)	(50)	(43)
- Atrophy	1	0	22	36	0	0	3	34
- Degeneration	0	5	9	1	0	3	10	0
MUSCLE, BICEPS FEMORIS	(47)	(48)	(45)	(39)	(43)	(47)	(50)	(42)
- Atrophy	0	0	20	32	0	0	3	32
- Degeneration	1	8	10	0	1	5	18	2
NERVE, SCIATIC	(44)	(47)	(46)	(38)	(46)	(42)	(49)	(43)
- Atrophy	0	0	0	8	0	0	0	5
- Axonal degeneration	10	9	37	25	7	4	32	33
NERVE, TIBIAL	(36)	(33)	(38)	(33)	(37)	(44)	(47)	(33)
- Atrophy	0	0	1	9	0	0	0	7
- Degeneration	7	12	30	14	7	2	26	14
SPINAL CORD, LUMBAR	(47)	(49)	(47)	(38)	(47)	(47)	(50)	(48)
- Axonal degeneration	21	24	40	25**	21	18	38	35
SPINAL CORD, THORACIC	(47)	(49)	(47)	(38)	(47)	(47)	(50)	(48)
- Axonal degeneration	6	7	17	11**	8	10	6	6
SPINAL CORD, SACRAL	(46)	(48)	(41)	(38)	(39)	(41)	(47)	(40)
- Axonal degeneration	0	3	5	7	0	0	3	9

( ) Total number examined

*Reprinted from pages 21 to 22 of report.

No increased tumor incidence over controls was found in any test group. Major causes of death reported among control and test groups ("when evident," i.e., as could be identified by the pathologist, as referenced in Appendix H of the report) were ascribed to chronic progressive nephropathy, pituitary adenoma, and malignant neoplasms, the frequencies and distributions of which were comparable in all groups.

Conclusions:

The authors conclude that the dietary administration of EPTC to CD rats at levels of 5, 25, and 125 mg/kg/day for 2 years caused the following compound-related effects: (1) Hindquarters

**The lower incidence in the high-dose group as compared to the mid-dose group may be related to decreased survival to term in the high-dose group.

neuromuscular disorder (both sexes), and externalized testes in mid- and high-dose animals, evident only during the second year; (2) discolored urine in high-dose males; (3) increased mortality in the high-dose male group; (4) dose-related decreases in body weight, (consistently) toxicologically significant at the mid and high doses, coupled with comparable reductions in food consumption; (5) a high percentage of cataracts among high-dose females, observed during the terminal week's ophthalmoscopic examination (and confirmed microscopically); (6) microscopic evidence of atrophy and axonal degeneration in sciatic and tibial nerves in mid- and high-dose males and females; (7) slightly, but not definitively, increased axonal degeneration at the lumbar and sacral levels of the spinal cord in these same test groups.

No compound-related changes were stated to have occurred in hematological or biochemical parameters, organ weights, or incidence of lesions (other than the tissue changes described above). No EPTC-induced neoplasia were recorded.

TB Evaluation/Core Classification:

The study appears to have been conducted in accordance with FIFRA Guidelines and with due regard to GLP conformity; quality assurance inspections were performed at regular intervals (Appendix A). No major discrepancies were found between individual animal data (Appendices B through H, J, and L) and summaries of group means (Tables 1 through 12). The conclusions stated in text by the authors about the effects "directly or indirectly attributable to the test article" (page 29) are supported by the tabulated data summaries and the individual animal data sheets appended. From the data presented in this study, it would appear that the LEL for EPTC-induced changes was 25 mg/kg/day (neuromuscular syndrome, body weight loss); consequently, the systemic NOEL may be set at 5 mg/kg/day. No induced tumor formation was found, even at levels causing increased mortality (i.e., exceeding the MTD).

The single major flaw in the final report is the failure in the text to discuss or comment upon the increased cardiopathy in high-dose males and females, identified microscopically as "chronic myocarditis multifocal, trace/mild/or moderate" in animals terminated at 12 months and 2 years (SAC) as well in those found dead or sacrificed in extremis (DOS) during the study (as found in Appendix H). Following is an enumeration of these cardiac findings extracted from Appendix H, summarized by group, sex, and fate (high dose and controls only*):

*As noted in Materials and Methods, a complete histopathology was not performed for the low- and mid-dose test groups, and thus heart slides (and those of most other major organs) were not routinely examined.

Incidences of Microscopic Cardiac Changes in Control and EPTC-Treated Rats (60/sex/group)*

Sex	Dose Group (mg/kg/day)	Dx**	Period of Study (months) 0 - 12 DOS	0 - 12 SAC	12 - 24 DOS	12 - 24 SAC	TOTAL CM DOS & SAC (%)
Males	0		[3]	[10]	[18]	[29]	23 (38.3)
		MCI	1	-	-	-	
		CM	-	-	4	15	
		MM	-	-	3	-	
	125		[11]	[10]	[25]	[14]	41 (68.3)
		MCI	3	-	-	-	
		CM	4	4	20	10	
		MM	-	-	-	-	
Females	0		[3]	[10]	[17]	[30]	6 (10.0)
		MCI	-	-	-	-	
		CM	-	-	1	4	
		MM	-	-	-	1	
	125		[2]	[10]	[24]	[24]	27 (45.0)
		MCI	-	-	-	-	
		CM	-	4	12	11	
		MM	-	-	-	-	
		AT	-	-	8***	-	

*Only heart slides from controls and high-dose EPTC animals were routinely examined (see Appendix H of the report).

**Dx, description of lesions (as given in Appendix H): MCI, mononuclear cell infiltration; CM, chronic myocarditis; MM, myocardial mineralization; AT, atrial thrombosis (organized).

[ ], Number examined.

-, all within normal limits.

DOS, died on study or in extremis during this period.

SAC, scheduled sacrifices (12 months; 2 years).

***All eight females with AT also had CM.

Prompted by the Agency's demand for ". . . submission of histopathology slides on the heart, muscle, hindlimb peripheral nerve, and spinal cord" (letter from PM Taylor to Riggs, December 17, 1984), all 480 animals were reexamined by the contracting laboratory (IRDC), and a report submitted by Stauffer.* However, this report contains only a rereading the heart slides, in order ". . . to confirm the incidence of heart lesions noted in the final report" (T-10001). In this reexamination by IRDC, the lesions were characterized by the following descriptive terms: Atrophy, chronic myocarditis, endocarditis, fibrosis, pericardial chronic inflammation, mineralization, pigmentation, and thrombosis. The incidence of cardiac lesions (all lesions of all degrees of severity from all animals), summarized from Table 1 of this addendum is as follows:

Sex	Dose Group (mg/kg/day)			
	0	5	25	125
Males (60/group)	24 (40.0%)	18 (30.0%)	26 (43.3%)	38 (63.3%)
Females (60/group)	8 (13.3%)	8 (13.3%)	7 (11.7%)	39 (65.0%)

In addition, it was noted that the degree of severity of chronic myocarditis (increased numbers of mild to moderate) was greater in test animals, especially evident at the high dose. Cardiac atrophy (reduced muscle mass, ventricular wall thinning, and reduction in fiber size), when present, accompanied chronic myocarditis; the greatest incidence of cardiac atrophy was found in high-dose females that died on study (48 percent, compared to 0 percent in controls).** It was reaffirmed in this Addendum

*"Addendum to the Final Report (Submitted September 30, 1983)" dated by IRDC April 23, 1985; submitted by Stauffer May 24, 1985, and given Accession Nos. 258076 and 260057 (both "Copy #2" of the same data).

**The increased myocardial atrophy in high-dose females is supported by increased enzyme activity of SGOT.

Report that absolute heart weights were reduced in high-dose animals (statistically, however, only for males sacrificed at termination, as given in the original report, T-10001).

The only other notable microscopic lesion was cardiac thrombosis, recorded in EPTC-treated rats predominantly dying on study with chronic myocarditis, and not in any control animal. Thrombosis was observed in one low-dose, one mid-dose and two high-dose males; however, more severe degrees of atrial as well as ventricular thrombi were recorded in high-dose females (eight atrial, four ventricular).

The authors propose no mechanism to explain the differences in incidences between male and female controls (as well as the low- and mid-dose EPTC groups).*

The addition of cardiopathy does not change the NOEL of 5 mg/kg/day for systemic effects ascertained from other compound-related changes, since significant increases in cardiac lesions were reportedly only observed microscopically at 125 mg/kg/day (the NOEL for heart lesions would then be set at 25 mg/kg/day). In addition to cardiac lesions at the highest dose, one also observes an increased axonal degeneration and muscle atrophy, increased cataracts in females, increased SGOT enzyme activity (which correlates with cardiac changes), decreased erythrocyte AChE activity, and decreased body weight gain and food consumption.

LEL = 25 mg/kg/day (decreased body weight gain, neuronal degeneration and muscular atrophy).

NOEL = 5 mg/kg/day

CORE CLASSIFICATION: MINIMUM for chronic toxicity
MINIMUM for oncogenicity

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* However, chronic myocarditis is commonly observed in older rats (F.A. Fairweather. Cardiovascular diseases in rats. In: E. Cotchin and F.J.C. Roe (Eds.), Pathology of laboratory rats and mice. Philadelphia: Davis, 1967), with age of onset of 13 months and older. A recent survey of age-associated lesions in Sprague-Dawley males by Anver and Associates (M.R. Anver, B.J. Cohen, C.P. Lattuada and S.J. Foster. Age-associated lesions in barrier-reared male Sprague-Dawley rats: A comparison between HAP:(SD) and Crl:COBS®-CD®(SD) stocks. Exper. Aging Res. 1982, 8:3-24) reported cardiomyopathy in 85 of 99 (85.9%) conventional Charles River CD's, and in 52 of 71 (73.2%) in the HAP-derived stock, increasing in prevalence with increasing age (from 59% for both stocks at 1 year, to 93 percent at 2 years).

[NB: In a previous 1-year feeding study providing levels of 0, 5, 20, and 80 (and 160) mg/kg/day Eptam Technical to Sprague-Dawley rats (Charles River stock) conducted for Stauffer by Hazleton Laboratories America, Inc.,* the following relevant changes were reported:

1. Clotting anomalies, with resulting increased incidence of splenic hematopoiesis/hemosiderosis (NOEL = 20 mg/kg/day);

2. Increased incidence of chronic myocarditis in high-dose animals of both sexes, considered by the authors (Hazleton) to be treatment related by virtue of exacerbating a condition occurring in "older rats."]

3. Increased SGOT levels at 80 mg/kg/day and 160 mg/kg/day in males and females.

* "54-Week Feeding Study in Rats (Subchronic Oral Toxicity)," dated September 18, 1978 (MRID 00022101); reviewed by Mitre July 6, 1983 as Document No. OPPT83W00205.

EPTAM

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